Haemophilia
The Official Journal of the World Federation of Hemophilia,
European Association for Haemophilia and Allied Disorders and
the Hemostasis & Thrombosis Research Society

have normal quantitative expression of glycoprotein Ib-IX-V complex. However, the glycoprotein can be functionally defective and therefore displays an identical phenotype to the classical form of the disease.

It is essential to maintain a broad differential diagnosis, especially when the clinical presentation is unusual, and does not respond to conventional therapies. Our findings demonstrate that, in cases of macrothrombocytopenia, reduced ristocetin-induced platelet aggregation may be sufficient for a diagnosis of Bernard–Soulier syndrome to be made even in the absence of a reduction in glycoprotein expression. The vast amount of SNP data, generated by genome projects will increasingly present interpretive challenges in assessing the variants of unknown clinical significance in specific patient populations. Further, the curation of SNP and locus specific mutation databases does not always favour the distinction between pathogenic and benign variants: In Bernard–Soulier syndrome, as in other autosomal recessive disorders, the report of a small number of heterozygous individuals should not preclude the variant from being considered potentially pathogenic in the homozygous state.

Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

References

1 Lanza F. Bernard-Soulier syndrome (hemorrhagiparous thrombocytic dystrophy). *Orphanet J Rare Dis* 2006; **1**: 46.
2 Kunishima S, Kamiya T, Saito H. Genetic abnormalities of bernard-soulier syndrome. *Int J Hematol* 2002; **76**: 319–27.
3 Kunishima S, Sako M, Yamazaki T, Hamaguchi M, Saito H. Molecular genetic analysis of a variant Bernard-Soulier syndrome due to compound heterozygosity for two novel glycoprotein Ibbeta mutations. *Eur J Haematol* 2006; **77**: 501–12.
4 Provan D, Newland A, Norfolk D *et al.* Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children and in pregnancy. *Br J Haematol* 2003; **120**: 574–96.

Replacement therapy in inherited factor VII deficiency: occurrence of adverse events and relation with surgery

M. NAPOLITANO,* A. DOLCE,† A. BATOROVA,‡ M. GIANSILY-BLAIZOT,§ J. INGERSLEV,¶ N. MIRBEHBAHANI,** M. N. D. DI MINNO,†† M. F. LOPEZ FERNANDEZ,‡‡ M. KARIMI,§§ P. CHAROENKWAN,¶¶ K. KAVAKLI*** and G. MARIANI†††

**Haematology Unit, Thrombosis and Hemostasis Reference Regional Center, Università di Palermo; †National Institute of Statistics, Palermo, Italy; ‡The National Haemophilia Centre, Institute of Haematology and Blood Transfusion, University Hospital, Bratislava, Slovakia; §Laboratory of Haematology, University Hospital, Montpellier, France; ¶Centre for Haemophilia & Thrombosis, University Hospital Skejby, Aarhus, Denmark; **Hematology and Oncology Research Center, Golestan University of Medical Sciences, Gorgan, Iran; ††Department of Clinical Medicine and Surgery, Federico II University, Naples, Italy; ‡‡Complejo Hospitalario Universitario A Coruña, A Coruña, Spain; §§Haematology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; ¶¶Division of Hematology, Chiang Mai University, Chiang Mai, Thailand; ***Children's Hospital, Ege University Faculty of Medicine, Izmir, Turkey; and †††Università di Ferrara Medical School, Ferrara, Italy*

The treatment paradigm for bleeding management in patients with congenital bleeding disorders is Replacement Therapy (RT), based on the substitution of the missing coagulation factor, with the aim of correcting the clotting defect. For the management of patients with factor VII (FVII) deficiency, a rare bleeding disorder, a number of therapies are available, including plasma or plasma-derived products as well as recombinant products [1,2]. However, information regarding optimal treatment schedules, treatment limitations and Adverse Events (AEs) is currently limited to anecdotal reports [3–8]. As a consequence, RT administration is influenced by factors such as the rarity of the disorder, the type, availability and supply of products, as well as economic and geographical factors.

Correspondence: Mariasanta Napolitano, Haematology Unit, Thrombosis and Hemostasis Reference Regional Center, Università di Palermo, Via del Vespro, 1- 97100, Palermo, Italy.
Tel.: (+39)0916554403; fax: (+39)0916554402;
e-mail: mariasanta.napolitano@unipa.it

Accepted after revision 3 July 2015

DOI: 10.1111/hae.12782

It is taken for granted that all RT types and schedules are associated with potential side-effects, and there is no doubt that the management of patients with bleeding disorders can be improved by reducing the occurrence of AEs and their severity. Awareness of AEs is indeed a relevant issue in the management of rare bleeding disorders, where data collection, especially in a prospective fashion, is very difficult.

The Seven Treatment Evaluation Registry (STER) has prospectively collected a large amount of information on the management of patients with congenital FVII deficiency [9–13]. The aim of this study was the systematic analysis of AEs related to treatments, for prophylaxis [9], surgical interventions [10,12] and spontaneous or traumatic bleeding episodes [11].

Patients with congenital FVII deficiency managed for a large array of clinical indications were investigated for RT-related AEs during the 8-year duration of the STER (deadlock date: February 28th, 2012). STER was a prospective, observational, multicentre, web-based registry created to collect and describe data on treatment modalities and outcomes in patients with this rare bleeding disorder. All treatment procedures were in accordance with the ethical standards and approved by the Ethics Committee of L'Aquila University Hospital (coordinator's institution) and the committees of all the other enrolling centres. Decisions on type of RT and schedules were autonomously taken by enrolling physicians.

Because of different viral ecologies, treatment attitudes and laboratory issues, pharmacovigilance was limited to non-blood-borne virus-related AEs; decisions on RT type and schedules were taken autonomously by the enrolling physicians. The STER study protocol was designed to capture the following elements related to AEs: clinical description, evaluation of severity (serious or non-serious), potential association with type of treatment (likely or unlikely) and outcome (including mortality for any reason). Thrombosis diagnosis (venous or arterial) was based on clinical suspicion and confirmed by imaging. FVII: c was determined at baseline and 15 min after the first RT administration for the treatment reported. STER protocol was designed to centrally determine inhibitors at the following time points: first enrolment (before RT administration), 30 days follow-up after the reported RT and when needed for clinical suspicion. Screening for inhibitory antibodies to FVII was conducted in a centralized fashion [4,13]; only baseline samples with FVII:c <4% were considered for inhibitor assay. Enrolling physicians were asked to carefully describe the type of AE, timing of occurrence after RT, clinical management and outcome. AEs were evaluated at the time of treatment and at the 30-day follow-up visit (or later if needed). Data were obtained from the STER database and analysed following a data quality and consistency check according to the STER data-management plan. We calculated the cumulative incidence rate of AEs in our cohort as the number of new cases per population at risk in a 8 years observational period and the incidence density rate (or person-time incidence rate) as number of new cases per population at risk in a given time period when the denominator is the sum of the person-time of the at risk population. With regard to every type of AE, we also calculated the prevalence in our cohort for specific adverse events as the proportion of cases in the population at the end of the study. Analyses were performed using the MEDCAL® software version 7.4.1.2 (http: www.medcalc.org). We here focus on inhibitor development and thrombosis, being these considered the most relevant AEs related to RT. Other side-effects reported after RT ($n = 11$) consisted mainly in re-bleeding ($n = 4$) and aspecific symptoms like headache, virosis and fever, judged as unlikely related to RT ($n = 7$). STER overall enrolled 225 patients with congenital FVII deficiency and a total of 312 treatments were administered: recombinant activated FVII (rFVIIa, Novo Seven®, Novo Nordisk A/S, Denmark, $n = 245$ [78.5% of all replacement therapies]); plasma-derived FVII concentrates (pd-FVII, Facteur VII™ LFB, Courtaboef, France or Provertin-UM TIM3™, Baxter, Vienna, Austria; FVII NF, Baxter, $n = 31$, 9.9%); Fresh Frozen Plasma (FFP), $n = 30$, 9.6%); Prothrombin Complex Concentrate (PCC, Prothromplex TIM 3, Baxter; Prothromplex Total TIM 4, Baxter, $n = 5$, 1.6%), and in one case FEIBA (Baxter). The AE's cumulative incidence was (2.2%, CI 0.6–3.9%) and the incidence density rate was 1.6% (CI 0.0–2.5%) per year. As for FVII inhibitors, 115 patients were centrally tested using a standardized modification in the Bethesda assay. Inhibitors occurred in two of those patients in whom a centralized screening could be done ($n = 125$); two other inhibitor patients were enrolled in the STER but inhibitor could not be centrally confirmed for logistical reasons. Three of the four reported inhibitors were detected during prophylaxis with rFVIIa, in two young boys at the age of 5 years and 1 year, respectively, and in one infant (a 5 months old male).

All cases had a very severe bleeding phenotype (epistaxis, easy bruising, gastrointestinal bleeding, CNS bleeding, subcutaneous and muscle haematoma). Baseline FVII:c levels were 2, 1.3 and <1% respectively. All the three patients were previously exposed to multiple FFP infusions and prophylaxis with rFVIIa was instituted to prevent CNS (in two cases) and GI (in one case) bleedings. The immune response in FVII deficiency was not complicated by anaphylactic reactions. Prophylaxis was continued in all three patients without any safety problem or reported dose-adjustment. All the patients were high responders with peak titres of between 34 and 68.3 BU mL^{-1}

Table 1. Thrombosis and inhibitors reported to the STER.

Sex/Age	Replacement therapy	FVII:c(%) 0′	FVII:c (%) 15′	Clinical setting	Event	Timing	Severity/Relation to replacement
Thrombosis							
M/8	rFVIIa 15 μg Kg^{-1} × 43, FFP 10 IU × 13	2.3	320	Neurosurgery	Cerebral infarct	8th day	Serious/Likely
M/23	rFVIIa 30 μg Kg^{-1} × 3	6	99	Orthopaedic surgery	SVT	1st day	Non-serious/ Likely
M/53	rFVIIa 30 μg kg^{-1} × 2, 22.5 μg kg^{-1} × 1, 15 μg kg^{-1} × 28	4.8	380	Renal transplant	Stroke	30 days	Serious/Likely
Inhibitors							
F/53* (p.A354V; p.464Hfs)	rFVIIa[†,‡] 30 μg Kg^{-1} × 1, 10 μg Kg^{-1} × 8	<1[§]	/	Minor surgery	High titre10–20 BU	Postoperatively	Serious/Likely[§]
M/5* (p.Ser112 Stop)	rFVIIa[†,‡] 30 μg Kg^{-1} × 21 days	2[§]	/	Prophylaxis for CNS bleeding	High titre 38-68.3 BU	3 months[¶]	Serious/Likely
M/0.5	rFVIIa[†,‡] 65 μg Kg^{-1} × 1/week	1.3[§]	/	Prophylaxis for CNS bleeding	High titre 5.5-60 BU	5 months[¶]	Serious/Likely
M/1	rFVIIa[†,‡] 31 μg Kg^{-1} × 3/week	<1[§]	/	Prophylaxis for GI bleeding	High titre 32–72 BU	1 month[¶]	Serious/Likely

Brackets report FVII gene mutation, SVT, superficial vein thrombosis. Age is expressed in years.
*inh. detected centrally.
[†]Schedule refers to RT prior to inhibitor(inh). detection.
[‡]All patients received different treatments (pdFVII, FFP) before inh. detection.
[§]Baseline levels.
[¶]Age at inhibitor screening.
BU=Bethesda Units.

(Table 1) at the last available follow-up (1 month after detection).

One case of inhibitor was detected in adulthood, in a 53 year-old woman previously treated with FFP, pd-FVII and PCC for recurrent bleeding episodes (mainly gynaecological). Inhibitor was discovered when the patient underwent multiple dental extractions, the patient was treated with rFVIIa given at an initial dose of 30 μg kg^{-1} followed by eight consecutive boluses of 10 μg Kg^{-1}. Patients were not reported to undergo any specific treatment for inhibitor eradication (Table 1).

Thrombosis occurred in three patients: two cerebral infarctions and one superficial vein thrombosis (1% of treatments). Dosing and treatment schedules are shown in the Table 1.

In the first case (M/8), treated for the evacuation of left cerebellar haematoma, RT with rFVIIa lasted 8 days; rFVIIa was administered immediately before the procedure and for the following 7 days at a dose of 18 μg Kg^{-1} every 6 h. FFP was than administered up to 16 days post procedure at a dose of 10 U Kg^{-1}, every 6 h.

In the second case (M/23), treated for knee replacement, RT with rFVIIa was given before the procedure and every 12 h in the following 24 h after orthopaedic surgery at a dose of 30 μg Kg^{-1}. Superficial Vein Thrombosis (SVT), confirmed with Doppler ultrasounds, involved the cephalic vein of the right arm.

In the third case (M/53), treated for kidney transplant, rFVIIa was administered 30 min before the procedure and 15 min thereafter. After 1 h, the dose of rFVIIa was empirically lowered to 22.5 μg kg^{-1}; after 4 h, rFVIIa was given at 15 μg kg^{-1}. The following RT administrations occurred at 8, 18, 24 and 30 h after the first one, namely at a doses of 15 μg kg^{-1}. Starting from 36 h, rFVIIa (15 μg kg^{-1}) was administered every 6 h until 96 h (day 4), when the same dose was injected twice a day for the following 6 days. The patient was discharged under immunosuppressive drugs. At 30 days follow-up visit he presented with a paralysis of the right leg started 48 h before admission, a CT scan confirmed the involvement of the left anterior cerebral artery and the patient was treated with Low Molecular Weight Heparin (LMWH) at prophylactic dose for 2 weeks, without any bleeding complication and a partial relief of paralysis. Further RT was not administered during LMWH.

Inhibitors to FVII were considered the most important side-effect associated with RT; we, therefore, performed a screening using a standardized modification in the Bethesda assay in a centralized fashion [4,6,13]. Of the two inhibitors screened and confirmed by the Central Laboratory, one had been previously detected; one was a *de novo* event. FVII gene mutation is available only for two patients with inhibitors (one with a missense mutation plus one codon deletion and another homozygous for a nonsense mutation).

Inhibitor occurrence in FVII deficiency can be considered at least as rare as that currently reported in haemophilia B [6], where a prevalence of 2–3% is accepted. Comparison to FIX deficiency is the most reasonable one, due to a high comparability in the gene and protein structures of FIX and FVII, even though severe gene defects, such as large deletions, have not been reported in patients with FVII deficiency [14]. Another substantial difference between the two bleeding disorders is the occurrence of anaphylactic reactions, a severe clinical issue in patients with haemophilia B, that is absent in patients with inhibitors to FVII, as confirmed by the fact that prophylaxis in FVII-deficient patients, can be continued even in the presence of an inhibitor [13]. Two other cases of inhibitor were reported in the STER but inhibitor detection could not be determined in a centralized fashion. Considering the total number of patients enrolled in the STER (n = 225), the prevalence (1.8%) appears unchanged.

The prevalence rate of thrombosis in the STER was 1% (3/312 treatments). This prevalence is the same as that found in a retrospective study by our group [5].

Postinfusion FVII coagulant activity levels did not differ between those patients experiencing AEs and the other uncomplicated treatments (data not shown), and no relationship could be established between thrombosis occurrence and the dose of rFVIIa or patient' age. The association between gene mutations and the risk of thrombosis has been explored [5] but even if two common mutations (Arg364Gln and Ala354Val) have been frequently detected in patients with thrombosis, no specific association has been found [5]. Thrombophilias, such as FV Leiden, prothrombin gene mutation, antiphospholipid antibodies syndrome and elevated clotting FVIII levels, were present in some cases of FVII deficiency with thrombosis, but they did not seem to play a relevant role as risk factors for thrombosis, since thrombophilia markers appeared to have a similar prevalence in patients with and without thrombotic complications [5]. This study suggests that FVII deficiency does not provide protection from thromboembolism, either arterial or venous, especially in a surgical setting. The indication for thromboprophylaxis in high-risk patients (e.g. patients undergoing major surgery and high-dose RT) still needs to be formally evaluated, but anecdotal reports suggest that it does not increase the risk of bleeding [5].

An important issue regards the frequency of surgery as a clinical setting associated with AEs, at least in this report (57.1%). Considering the reported AEs and the other side-effects (n = 11, data not shown), surgical interventions appear to play a not negligible role in the occurrence of adverse events (RR 2.5, P = 0.0574, CI 0.97–6.54).

This study focusing on AEs checked prospectively in a large number of patients with FVII deficiency and over a large array of clinical settings highlights a low-to-very-low incidence of AEs. This indicates a very good safety-to-efficacy ratio, especially for rFVIIa, the most used replacement treatment.

Acknowledgements

The authors thank PAREXEL for editorial assistance with the manuscript.

Author contributions

M. Napolitano and G. Mariani prepared the drafts and interpreted the data. A. Dolce maintained the database, performed the data analysis and generated the figure. A. Batorova, M. Giansily-Blaizot, N. Mirbehbahani., M.N.D. Di Minno, M.F. Lopez Fernandez, M. Karimi, P. Charoenkwan and K. Kavakli collected data, contributed to writing the results section of this manuscript, enrolled patients and collected samples. J. Ingerslev performed centralized determination of inhibitors and critically revised the methods section. GM, the STER Study Group coordinator, critically revised data and revised all the text versions. All authors have revised and approved the manuscript.

Disclosures

MG-B has occasionally received lecture fees from Novo Nordisk. JI has been an occasional speaker for Novo Nordisk at training courses, and his institution has received a grant for serving as a central laboratory on behalf of STER. PC and KK received research support from Novo Nordisk for this study. GM has received consultation fees or honoraria, lecture fees and research support from Novo Nordisk. MN, AD, AB, NM, MNDDM, MFLF, MK have no conflicts of interest to declare. Editorial assistance to the authors during the preparation of this manuscript was provided by Sharon Eastwood (medical writer, PAREXEL) and financially supported by Novo Nordisk A/S, in compliance with international guidelines for good publication practice.

References

1 Mariani G, Bernardi F. Factor VII deficiency. *Semin Thromb Hemost* 2009; **35**: 400–6.
2 Mariani G, Dolce A, Marchetti G, Bernardi F. Clinical picture and management of congenital factor VII deficiency. *Haemophilia* 2004; **10**: 180–3.
3 Mariani G, Testa MG, Di Paolantonio T, Molskov Bech R, Hedner U. Use of recombinant, activated factor VII in the treatment of congenital factor VII deficiencies. *Vox Sang* 1999; **77**: 131–6.
4 Ingerslev J, Christiansen K, Sørensen B; International Registry on Factor VII Deficiency (IRF7) Steering Committee. Inhibitor to factor VII in severe factor VII deficiency: detection and course of the inhibitory response. *J Thromb Haemost* 2005; **3**: 799–800.
5 Mariani G, Herrmann FH, Schulman S, Batorova A, Wulff K, Etro D *et al.* Thrombosis in inherited factor VII deficiency. *J Thromb Haemost* 2003; **1**: 2153–8.
6 Mariani G, Konkle B, Kessler CM. Inhibitors in hemophilia A and B. In: Hoffman R, Benz EJ Jr, Silbertein LE, Heslop HE, Weitz JI, Anastasi J. Hematology: Basic Principles and Practice, 6th ed. Chapter 138, Philadelphia, PA: Saunders, 2012.
7 Pruthi RK, Rodriguez V, Allen C, Slaby JA, Schmidt KA, Plumhoff EA. Molecular analysis in a patient with severe factor VII deficiency and an inhibitor: report of a novel

mutation (S103G). *Eur J Haematol* 2007; **79**: 354–9.
8 Brenner B, Wiis J. Experience with recombinant-activated factor VII in 30 patients with congenital factor VII deficiency. *Hematology* 2007; **12**: 55–62.
9 Napolitano M, Giansily-Blaizot M, Dolce A *et al.* Prophylaxis in congenital factor VII deficiency: indications, efficacy and safety. Results from the Seven Treatment Evaluation Registry (STER). *Haematologica* 2013; **98**: 538–44.
10 Mariani G, Dolce A, Batorova A *et al.* Recombinant, activated factor VII for surgery in factor VII deficiency: a prospective evaluation - the surgical STER. *Br J Haematol* 2011; **152**: 340–6.
11 Mariani G, Napolitano M, Dolce A *et al.* Replacement therapy for bleeding episodes in factor VII deficiency, A prospective evaluation. *Thromb Haemost* 2013; **109**: 238–47.
12 Mariani G, Dolce A, Napolitano M *et al.* Invasive procedures and minor surgery in factor VII deficiency. *Haemophilia* 2012; **18**: e.63–5.
13 Batorova A, Mariani G, Ingerslev J *et al.* Inhibitor of FVII in congenital severe factor VII deficiency. *J Thromb Haemost* 2007; **5**: P-S-199.
14 Mariani G, Herrmann FH, Dolce A *et al.* Clinical phenotypes and factor VII genotype in congenital factor VII deficiency. *Thromb Haemost* 2005; **93**: 481–7.

Bilateral upper limb compartment syndrome induced by strenuous exercise in a patient with haemophilia A and a low titre inhibitor

M. A. ABDELHALIM,* C. R. SHAW,* Z. ABU AL-RUB,* D. HOPPER,† J. P. HANLEY,† K. L. TALKS,† T. T. BISS† and P. V. FEARON*
**Department of Trauma & Orthopaedics, Royal Victoria Infirmary, Newcastle, UK; and †Department of Haematology, Royal Victoria Infirmary, Newcastle, UK*

Compartment syndrome is a surgical emergency caused by an intrinsic or extrinsic event leading to an increased tissue pressure within a closed fascial compartment. Once this exceeds perfusion pressure a number of serious complications can occur with microvascular compromise and eventual tissue necrosis. Whilst the vast majority of cases of compartment syndrome result from fractures secondary to trauma, individuals with haemophilia present a unique group of patients at risk of spontaneous bleeding into a fascial compartment or joint [1]. We present a case of atraumatic bilateral compartment syndrome of the forearms in a 21-year-old male with severe haemophilia A and a FVIII inhibitor.

A 21-year-old man with a background of severe congenital haemophilia A was admitted with pain and swelling of his left forearm. He had had a recent brief admission with a left shoulder haemarthrosis which was treated with rFVIII. Clinical response was poor and reduced FVIII half-life was demonstrated following which the presence of a factor VIII inhibitor, with a titre of 1.0 BU mL^{-1}, was confirmed. Treatment with recombinant factor VIIa (rFVIIa, NovoSeven®; Novo Nordisk A/S, Bagsværd, Denmark) was commenced. He had no other significant past medical history. There was no history of direct trauma, although he had been attending the gym daily over a 2-month time period for weights training and body building. He admitted using protein shakes, purchased over the internet, but denied use of anabolic steroids. A rapid increase in his upper body muscle bulk had been recently noted by the haemophilia physiotherapist.

On admission, he was haemodynamically stable but had pain on wrist and finger flexion and an area of erythema around a previous cannulation site on the medial aspect of his left forearm. He was treated with intravenous antibiotics for cellulitis and rFVIIa for possible haematoma. An ultrasound scan performed to investigate progressive forearm swelling identified external cephalic vein thrombosis but no evidence of haematoma. Despite ongoing treatment, the symptoms progressed and pain became uncontrollable with opiate analgesia. Active finger movements became impossible. Passive extension caused severe pain and the forearm muscle bellies were exquisitely tender. However, radial and ulnar pulses were present and sensation was intact. A clinical diagnosis of compartment syndrome was made by the orthopaedic team and the patient was taken to theatre for urgent left forearm superficial and deep flexor compartment decompression. Compartment pressures were not measured. The muscles of the forearm were found to be grossly swollen with evident intramuscular bleeding, but no focal haematoma. Tissue necrosis was not evident at the

Correspondence: Mohamed A. Abdelhalim, Department of Trauma & Orthopaedic Surgery, Royal Victoria Infirmary, Newcastle NE1 4LP, UK.
Tel.: +44 7875274554;
e-mail: ma.abdelhalim@gmail.com

Accepted after revision 3 July 2015

DOI: 10.1111/hae.12783